III. REMARKS

Claim Status

Claims 1-2, 6-13 and 18-34 are under current examination. Claims 1-2, 6-13 and 18-34 stand rejected.

Claim Rejections - 35 USC § 103

Claims 1-2, and 10-13 and claims 1-2, 6-13 and 18-34 are rejected under 35 U.S.C. 103(a) as being obvious over Margolskee (US-5,817,759, issued 6 October 1998) in view of Yao et al. (US-7,041,457, issued 9 May 2006) and further in view of Ruiz-Avila et al. (PNAS. July 17 2001. vol.98; No.15: 8868-8873).

a) Prosecution History

In a previous office action the examiner rejected Claims 1-6 and 9-17 under 35 U.S.C. 103(a) as being obvious over Margolskee (USP 5,817,759) in view of Yao et al. (USP 7,041,457).

Applicant's response to the previous office action overcame this rejection which was expressly withdrawn. Applicant, in the unentered amendment, limited his argument to the newly cited reference since the rejection over the first two references had already persuaded the examiner.

The examiner attempts to dismiss the applicants argument as spurious, stating:

> "It is the combination of references which makes the instantly claimed invention obvious (as recited above in the text of the rejection). The applicant's arguments are spurious and unpersuasive."

Applicant is fully cognizant of the necessity of addressing the combination of references rather than each reference singly. Applicant expected that the examiner would be aware that the record in this application showed that the applicant had already addressed the combination of Yao et al. and Margolskee in a prior office action, in response to which the examiner withdrew the rejection, and did not expect that the examiner would require a reiteration of an argument that had already persuaded the examiner that he had not made out a *prima facie* case of obviousness.

Since there must be, as a matter of logical necessity, some disclosure present in the third reference, that was lacking in the first two references, applicant addressed the third reference and argued that the third reference did not fill the hole in the examiner's argument, a hole acknowledged by the examiner, in withdrawing the rejection over the first two references.

Thus, rather than repeat the arguments which persuaded the examiner not to renew the rejection, applicant addressed how and in what way the addition of the new reference to the combination might cure the lacuna in the examiners argument.

b) Summary of Argument

Applicant discloses and claims a $G_{\alpha q\text{-Gustducin}}$ chimeric G-protein wherein the last 44 amino acids of the $G_{\alpha q}$ protein sequence are replaced with a 44 amino acid unit of Gustducin, where such 44 amino acid unit of Gustducin is the last 44 amino acids of SEQ ID NO:2.

Margolskee et al. do not disclose a chimeric protein but merely Gustducin, Yao et al. disclose chimeric proteins comprised of different units ('mouse' Gaq rather than 'human' Gaq, and transducin rather than gustducin) which has a low 58% homology to the claimed proteins and Ruiz-Avila et al. do not disclose a chimeric G protein, but instead disclose a specific single base mutant of gustducin.

Thus, in combination, the prior art discloses Gustducin, (Margolskee et al.) a mutant of gustducin with a specific single base modification (not a chimera) (Yao et al.) and chimeric proteins comprised of 'mouse' (not human) Gaq and transducin which have a low 58% homology to the claimed proteins.

A one skilled in the art, the examiner is aware that proteins with merely 58% homology cannot be expected to function in the same manner. This is highlighted by the prior art (Ruiz-Avila et al.) which further states that a single base modification limits responsiveness.

Thus, the protein of Ruiz-Avila et al. cannot be utilized to render obvious a different protein

The Examiner also asserts that the applicant has not pointed out why the references are not combinable. Applicant apologizes if the point was not set forth with sufficient specificity in the prior response.

Applicant believe that using Ruiz-Avila et al. in combination does not add anything relevant to the previously combined references of Margolskee et al. (who merely discloses Gustducin) and Yao et al. (who discloses various 'different' chimeric proteins based on 'different' units that are only 58% homologous to the claimed proteins), when used in combination. Only Yao et al. is directed to chimeric proteins, so Margolskee et al. and/or Ruiz-Avila et al. when combined with Yao would not have led the artisan to expect success in making 'different' (only 58% homology) chimeric proteins based on 'different' basic units that are functional and useful in a screen.

Of crucial importance, Ruiz-Avila is directed to α-Gustducin, and reports in the title of this journal article that a 'single' mutation causes a loss of responsiveness to sweet and bitter compounds. Applicant respectfully suggests that this demonstrates beyond any reasonable doubt that an only 58% homologous construct would not be expected to retain basic functionality let alone retaining such functionality to such a degree as to allow its use for taste receptor screens?.

As the examiner is aware, the field of molecular biology is a highly unpredictable one so it is impossible to know beforehand, and is therefore non-obvious in the absence of impermissibe hindsight, to know whether a new chimeric protein would be correctly transcribed, folded and functional.

In addition, it is even more unpredictable to know whether the resulting signal would be high enough to allow a screening assay to work. Notably, this is a particularly relevant issue with taste receptor screens which have notoriously low signals, as set forth in the instant specification:

> "However, there are many technical challenges in developing reliable assays based on an heterologous expression systems. One problem resides in the reliable expression of high concentrations of GPCRs at the surface of a foreign host cell. A second problem is the provision of G-proteins that not only are able to couple with many different types of GPCRs (often, such G-proteins are referred to as “promiscuous”), but also couple with high efficiency, such that even with relatively low surface concentrations of GPCRs, the cell signal is as strong as possible.")[published specification, para. 0005]

The Examiner acknowledges that Yao et al. do not specifically teach making a chimera between a Gq protein and gustducin. To bridge this hole in the art, the examiner asserts that it is "clearly obvious" in light of the teachings of the art involving substitutions with C-terminal sequences from other chemisensory proteins such as transducin.

Applicant respectfully disagrees and points to the very art cited by the examiner. As Ruiz-Avila et al. demonstrate, even a single mutation may make a huge difference, in the latter case, the loss of responsiveness. Ruiz-Avila et al. demonstrate that it anything but clear how any system, without additional information, will react to substitutions and the Examiner has not indicated any such information that would lead the skilled

artisan expect functionality or suitability for taste receptor screening.

Applicant believes the above discussion should overcome the examiner's concern that "The applicant has not pointed out why the references are not combinable."

Furthermore, the examiner states that applicant has not pointed out which limitations are not taught by the prior art.

The examiner has explained (1) Yao et al. teach a chimeric Gq-protein wherein the C terminus is replaced by 44 amino acids of transducin; (2) Margolskee specifically teach the 40 amino acid carboxy terminal end of gustducin and the homologies between the C-termini of gustducin and transducin; and (3) Ruiz-Avila et al. teach that the interaction of gustducin with its cognate taste receptors is similar to that of transducin with rhodopsin.

The examiner concludes that together these references render obvious a chimeric Ga protein wherein the 44 amino acid C-terminus comprises the c-terminal 44 amino acids of gustducin.

Applicant respectfully disagrees with this line of argumentation. At best the references, assuming *arguendo* that one skilled in the art would be motivated to combine the references, suggest an extensive research program to discover whether there exists a chimeric G protein of some sort having some minor degree of homology to transducin or gustducin at its c-terminal end. Even so, the combination of references would lead one to modify only one amino acid at a time, following the headlined title of Ruiz-Avila et al. that even a single modification modified responsiveness.

In conclusion, applicant points out that the prior art does not disclose this chimera or any other chimera that is closely related to the claimed chimera . Applicant respectfully suggests this the reference are to far afield to provide a *prima facie* case of obviousness.

c) further analysis of prior art

All the claims in the application are rejected under 35 U.S.C. 103(a) as being obvious over Margolskee (US-5,817,759, issued 6 October 1998) in view of Yao et al. (US-7,041,457, issued 9 May 2006) and further in view of Ruiz-Avila et al. (PNAS. July 17 2001. vol.98; No.15: 8868-8873).

Claim 1 is directed to a G_{aq} Gustducin chimeric G-protein where the last 44 amino acids of the $G_{\alpha q}$ protein sequence are replaced with a 44 amino acid unit of Gustducin.

Applicant has recognized a problem not known or suggested by the cited references and solved that problem in the novel manner described in the instant specification.

Applicant highlights the problem solved by the instantly claimed invention at page 3 of the specification:

> "Thus, since sweet, umami and bitter taste receptors are believed to couple to Gustducin (a Gam type G-protein) and the aforementioned studies suggest that, Ga_{15} and $Ga16$ are not the optimal partners for G_{am} type GPCRs, a skilled person would not expect efficient coupling of activated taste receptors to either $Ga15$ or G_{a16}. On the other hand, Gustducin itself does not offer a practical solution as this G-protein modulates release of cyclic nucleotides (cNMPs) which are not as easily measured as, for example, increases in Calcium ions. It is technically difficult to measure cNMPs and requires an immunoassay that generally takes in the

> order of 4 to 6 hours, and then only provides an end-point assessment. Still further, it is complicated to use a specialized G_{ai} protein, such as Gustducin in a heterologous cell expression system. To do so, one would have to introduce two additional G-protein sub-units (the beta and gamma sub-units) into the heterologous host cells to fully reconstitute the taste-receptor- G-protein complex. G_{a16} on the other hand can complex with beta/gamma sub-units endogenous to mammalian cells, such as cells of the HEK 293 cell line. It is faster, easier and more sensitive to employ G_{a16} rather than G_{ai}-type of G-protein such as Gustducin."

The solution to the problem is set forth on page 4 of applicant's specification:

> "Surprisingly we have now found that chimeric G-proteins based on Gaq-Gustducin are able to bind to a wide range of known and putative bitter taste receptors, and sweet and umami receptors with high affinity.
>
> Accordingly the invention provides in a first aspect a Gaq-Gustducin chimeric G-protein.
>
> In a specific embodiment the chimeric $_{Gaq-Gustducin}$ is a Ga_{15} or $_{16-Gustducin}$ protein, more specifically a $G_{a16-Gustducin}$ protein."

The references do not disclose or suggest the claimed protein. Margolskee does not disclose chimeric proteins. Margolskee merely discloses the known protein Gustducin, in particular the polynucleotide sequences encoding the alpha subunit of Gustducin or fragments of variants.

Margolskee discloses the Gustducin protein and discloses that activation of inhibition of the α subunit of Gustducin modifies perceived taste. The examiner recognizes that Margolskee does not teach the G_{aq}-Gustducin chimeric G-protein and also does not recite replacement of the C-terminal sequence of Gustducin with 5-44 amino acids of the Gustducin receptor.

Yao et al. is cited as teaching various G_{aq} chimeric G-proteins including Gαq and Gα15 (in mice, Gα16 in humans) and in particular, that the chimeric promiscuous or widely promiscuous Gq proteins described in Yao et al. may have sequences incorporated from other Gα class proteins.

Yao et al. discloses chimeric proteins based on Gaq (in particular, various mouse variants, compare table I) combined with the 5 last amino acids of either transducin or GaOLF. Yao specifically discloses chimeric proteins based on mouse Ga15 having the last 5 amino acids replaced (several variants).

Yao et al. is also cited as teaching chimeric Gq variants and the isolated nucleic acids encoding the same. In one embodiment disclosed by Yao et al. the chimeric Gq protein variants comprise C-terminal sequences from transducin, which is stated as exhibiting improved functional coupling to taste receptors.

While Yao discusses hypothetical chimeric proteins comprising fragments of up to 44 aminoacids of transducin and G_{aolf}, it is not clear whether those will work at all. The field is highly unpredictable, and many possibilities of choosing chimeric G-Protein combinations exist. There is no motivation in Yao et al. to choose the 44 amino acid long variant over any other (actually, 5 amino acids are exemplified), nor is there any motivation to choose a particular G-Protein of the various ones that are suggested, which notably excludes gustducin.

The examiner states that Yao et al. further teaches substituting from 5 to 44 amino acids from the C terminus of transducin or G_{aolf}. The examiner therefore concludes that substituting Gustducin for transducin or G_{aolf} would be obvious.

Yao et al. does not mention Gustducin at all, while it does discuss the "gustducin-coupled bitter receptor mT2R5" as a Gaq variant. Yao does not disclose any chimeric proteins that comprise a 44 amino acid fragment of gustducin.

There is no prior art reference that teaches which parts of which proteins to combine to arrive at a high affinity G-Protein that, thanks to the gustducin-part, couples not only to bitter, but also sweet and umami receptors, thus being a clear advance over the prior art.

Ruiz-Avila et al. is cited by the examiner as disclosing the importance of the C-terminus for interacting with taste receptors. But this is already disclosed by Yao et al. In Yao et al.'s claim 1 the claim requires the replacement of at least 5 amino acid residues from the C-terminus of a mutated Gq protein. Even were this disclosure not present in Yao et al., Ruiz-Avila gives no inidication of which amino acid resideues to modify and in fact, teaches that modification can hinder responsiveness. Yao et al. state, in their specification:

> For instance, the present inventors have also discovered that the Gly to Asp mutation is synergistic with the replacement of the C-terminus of $G\alpha_q$ by that of transducin or $G\alpha_{olf}$. $G\alpha_q$ proteins containing C-terminal amino acids from transducin or $G\alpha_{olf}$ in combination with a Gly66 to Asp alteration show increased activity compared to individual chimeras alone. A preferred embodiment is a variant G_q proteins having at least about five amino acids in the C terminus of said G_q protein replaced by at least about five amino acids from the C terminus of $G\alpha_{olf}$, if or transducin, wherein said C-terminal substitution increases promiscuity of said variant G_q protein as compared to the corresponding native G_q protein. Up to 44 amino acids of the C terminus of transducin or $G\alpha_{olf}$ may be incorporated. Other possible variants are shown

in FIGS. 3 and 4.

Thus, the disclosure of Yao et al. already shows the importance of C-terminal amino acids and this aspect of Ruiz-Avila et al. is merely additive to Yao et al. The examiner, having already acknowledged that applicants have overcome the combination of Yao et al. and Muskologee in the prior action, has not made out a new *prima facie* case or added any new disclosure.

Yao et al. is directed to chimeric proteins and discloses a Gaq-transducin44 chimeric protein that is 58% homologous to the G16gust44 chimeric protein of the invention i.e. very much different from the claimed chimeric G-Proteins which are 90% homologous to SEQ ID NO:2.

Margolskee merely discloses Gustducin, but no chimeric G-proteins. Ruiz-Avila et al. is also not directed to chimeric G-proteins either, and does not disclose the suitability or interchangeability of either transducin or gustducin in a chimeric G-protein.

Ruiz-Avila et al. exclusively addresses the natural interaction of gustducin and, commenting on various studies, mentions that the latter suggest that the interaction of gustducin with its cognate taste receptors, a key determinant of which is its C-terminus, may be similar to that of transducin with rhodopsin. Notably, the latter does not suggest anything in terms of actual functionality of one or the other partial protein in a chimeric protein, neither correct folding, coupling efficiency of the G-protein to the chimeric receptor nor resulting signal strength. This is significant in light of the fact that the **native** versions of the claimed G-proteins (G15,

G16 and their homologs) do not couple effectively, in contrast to the **chimeric** G-proteins that are claimed.

Therefore, the addition of Ruiz-Avila as a third document does not disclose information that would allow one skilled in the art to arrive at the invention - the skilled person finds no direction whatsoever on which components may be combined in which way to result in a fully functional chimeric G-protein, much less a chimeric G-Protein with an improved coupling efficiency.

An artisan would therefore not be motivated to make the claimed chimeric G-Protein, nor would have expected success based on Yao et al.'s chimeric G-Proteins, which share only about 60% homology with the claimed chimeric Gaq-gust44-Proteins.

Applicants believe the above explanations are sufficient to negate any *prima facie* case of obviousness and respectfully request favorable reconsideration and allowance of these claims.

Please charge any insufficiency of fees, or credit any excess, to Deposit Account No. 14-1263.

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